The signaling pathways involved in the synergistic effect of ET-1 and cAMP on IL-6 transcription

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We demonstrated previously that ET-1 and cAMP may synergistically induce IL-6 release from adipocytes, mainly through their strong stimulatory effect on IL-6 gene expression. In the present study, we further examined the signaling pathways that may be involved. A luciferase reporter driven by promoter (−1310/+198) of mouse IL-6 gene was transfected into 3T3-L1 adipocytes to monitor IL-6 transcription in response to ET-1 and 8-bromo cAMP, and the effects of various inhibitory agents were tested. Whereas the stimulatory effect of ET-1 alone was inhibited by pertussis toxin (PT), GF109203X, U0126, N-acetylcysteine, salicylate, dominant negative CREB (dn- CREB) and mithramycin A, the stimulatory effect of 8-bromo cAMP was only inhibited by dn-CREB. On the other hand, the synergistic effect of ET-1 and cAMP was suppressed by GF109203X, U0126, salicylate, c-Jun-specific antisense oligonucleotide (AS-cJun) and dn-CREB. PT had a partial inhibitory effect, while NAC and mithramycin A had no influence. Since NF-kB activation by ET-1 is mediated by a PKC&epsilon/ROS cascade, the observation that the synergistic effect of ET-1 and cAMP was inhibited by salicylate but not NAC suggests salicylate inhibited some factor in addition to NF-kB. Indeed, we found that another salicylate target p90 ribosomal S6 Kinase (RSK) was involved. Taken together, the synergistic effect of ET-1 and cAMP on IL-6 gene transcription seems to be mediated by pathways involving PKC, MAPK, CREB, AP-1 and RSK.

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A novel mouse model to characterize the mechanisms of endothelin-1-induced vascular injury

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Background: To develop a model to study of the mechanisms of endothelin-1 (ET-1)-induced vascular injury, we generated an inducible endothelial cell (EC)-restricted human ET-1 (EDN1) cDNA overexpressing transgenic mouse. Methods and results: A transgene was engineered that expresses chloramphenicol acetyltransferase (cat) and EDN1 cDNA prior and after Cre-mediated excision, respectively, under the control of cytomegalovirus immediate early enhancer/chicken β-actin promoter (CAG). Co-transfection of Cre expression vector and pCAG-cat-EDN1 caused 30-fold increase in ET-1 production. Transgenic mice were generated and two of seven founder lines were selected based on their cardiac cat expression level, which was twice higher in line C-134 than C-170. Inducible EC-restricted EDN1 mice were generated crossing CAG-cat-EDN1 mice with transgenic mice expressing CreERT2 under control of EC-specific Tie2 promoter. To investigate the extent of CreERT2 activation by tamoxifen and tissue specificity, Tie2-CreERT2 mice were crossed with ROSAmT-mG/mT-mG reporter mice expressing a membrane-targeted tandem dimer tomato (mT) before Cre-mediated excision, and membrane-targeted enhanced green fluorescent protein (mG) after excision. Tie2-CreERT2/cat-EDN1 and Tie2-CreERT2/ROSAmT-mG/+ mice were treated subcutaneously with 1 mg tamoxifen/day for 5 days and sacrificed 14–16 days later. mG expression was detected in 22 ± 3% of mesenteric artery ECs of Tie2-CreERT2/ROSAmT-mG/+ mice. Plasma ET-1 levels were similar in vehicle-treated Tie2-CreERT2/cat-EDN1 (1.2 ± 0.1 pg/mL, n = 4) and wild-type mice (1.1 ± 0.1 pg/mL, n = 2), demonstrating no leaky expression. Tamoxifen induced 8-fold increase in plasma ET-1 levels in Tie2-CreERT2/cat-EDN1 mice (9.1 ± 0.3 pg/mL, n = 4). Conclusion: Tie2-CreERT2/cat-EDN1-inducible EC-restricted EDN1 overexpressing mice allow the study of ET-1 vascular effects independently of developmental effects.

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Remodeling of endothelial function in atherosclerotic mice overexpressing endothelin-1 restricted to endothelium

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In human atherosclerosis, which is associated with elevated plasma and coronary endothelin (ET)-1 levels, ETA receptor antagonists improved coronary endothelial function. Mice overexpressing ET-1 specifically in the endothelium (eET-1) crossed with apolipoprotein E knockout mice (Apoe −/−) exhibited exaggerated high-fat diet (HFD)-induced atherosclerosis. Since endothelial dysfunction often precedes atherosclerosis development, we investigated whether endothelium-specific ET-1 overexpression causes endothelial dysfunction in Apoe −/− mice. Male 8-week old eET-1, Apoe −/−, eET-1/Apoe −/− and wild-type mice were fed a regular diet or HFD for 8 weeks. Endothelial function was assessed in mesenteric arteries by pressurized myography. In HFD-fed mice, acetylcholine-induced endothelium-dependent relaxation (EDR) was reduced 67% in Apoe −/− and 41% in eET-1 compared to wild-type ($P < 0.05$). Surprisingly, EDR was not impaired in eET-1/Apoe −/− compared to wild-type. Endothelium-independent relaxation to nitric oxide donor sodium nitroprusside and contractile responses to norepinephrine were unaffected. Similar results were observed in regular diet-fed mice. In the presence of inhibitors of either nitric oxide synthase (NOS)-mediated relaxation, Nω-nitro-l-arginine methyl ester, or endothelium-dependent hyperpolarization (EDH)-mediated relaxation, apamin plus Tram34, EDR was blunted in wild-type ($P < 0.01$), whereas NOS-mediated relaxation was reduced 22% ($P < 0.05$) and EDH-mediated relaxation unaffected in eET-1/Apoe −/−. However, the concomitant inhibition of NOS- and EDH-mediated relaxation reduced EDR 51% in eET-1/Apoe −/− ($P < 0.05$). These results show an interdependence of NOS and EDH pathways in EDR in wild-type mice. ET-1 overexpression induced development of compensatory mechanisms in pre-atherosclerotic arteries of Apoe −/− mice, which permits either NOS or EDH pathway to mediate EDR independently. Investigation of mechanisms involved in EDR remodeling in eET-1/Apoe −/− mice will allow a better understanding of the ET-1 role in atherosclerosis.

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